KANSAS UNIVERSITY SCIENCE BULLETIN.

VOL. IV, No. 14. SEPTEMBER, 1908. { WHOLE SERIES, VOL. XIV, No. 14.

ORGANIZATION OF THE CHROMOSOMES IN PHRYNOTETTIX MAGNUS.

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(Contribution from the Zoölogical Laboratory, No. 181.)

Plates XXIII, XXIV.

CYTOLOGICAL investigations in this laboratory on the spermatogenesis of various *Acrididæ* have resulted in discoveries of primary importance concerning the precision in the organization of the *Acrididæan* chromosomes. McClung, in a comparative study of "The Chromosome Complex of Orthopteran Spermatocytes,"* finds that a "definite series of chromosomes" occurs in all the members of the family, and that the modifications in size, form and association of these elements accompany variations in somatic characters that account for the division of families into genera and species. Striking examples of the constancy of such modifications were found in the multiple chromosomes of *Hesperotettix* and *Mermiria,* which he describes. In establishing the individuality of the chromosomes different workers have found these elements exhibiting constant characteristics of size, form and function.

In my brief study of the male germ-cells of *Phrynotettix magnus* I have found further convincing evidence of a precise and definite order governing the cell processes exhibited in the internal organization of the chromosomes. The work was done under the direction of Doctor McClung. The investigations of McClung on the "Spermatocyte Divisions of the *Acrididæ*"† and Sutton on the "Spermatogonial Divisions of *Brachy-*

* Biological Bulletin, vol. 9, No. 5, 1905.

† Kansas University Quarterly, vol. 9, No. 1, 1900.

stola magna"* make a detailed account of the manner of these divisions in this species unnecessary, since in my material the divisions display the typical order of the *Acrididæ*.

SPERMATOGONIA.

A polar view of the spermatogonial chromosomes of *Phrynotettix magnus* shows twenty-three chromosomes appearing as longitudinally split rods of varying lengths (fig. 1). The accessory chromosome (x) can be distinguished from the others by its rough contour and the more marked hyaline area surrounding it. In the succeeding anaphase, division is accomplished by a separation of the halves of the chromosomes. Separation proceeds from the proximal to the distal end and results in the proximal ends reaching the poles first. The same peculiarities that distinguished the accessory in the metaphase are also observed in this stage (fig. 2 x). During the anaphase the cell elongates in the direction of the spindle axis. The chromosomes are drawn close to the opposite ends of the elongating cell and, on account of their number and relative thickness, lie approximately parallel to each other (fig. 3). The spindle between the two groups of chromosomes which are to form the nuclei of new cells persists between the daughter-cells and fades away near the groups of nuclear elements. The subsequent characteristic changes through which the spindle remains pass form an excellent criterion in determining the sequence of the various changes which are observed in the cell. As the division wall is formed the spindle is slowly contracted. What Sutton describes as a tendency of the daughter-cells "to roll upon one another" is plainly seen from figure 4 to be a rolling of the nucleus, which forces the spindle fibers to one side of the cell. The same figure shows this peculiar movement of the nucleus to be accompanied by a disintegration of the chromosomes, which, together with the persistence of the spindle fibers, may bear a causal relation to the former phenomenon.

As the separation of chromatin granules begins the chromosomes move apart, retaining, however, their parallel arrangement, and each chromosome becomes surrounded by a delicate membrane forming the chromosomal vesicle. The dissociation of chromomeres in the telophase results in the diffusion of the chromatin within the vesicles, which, so far as I have

* Kansas University Quarterly, vol. 9, No. 2, 1900.

been able to ascertain, remain intact until an early prophase. In these stages also the accessory chromosome is readily recognized by its position, size and apparently advanced state of diffusion, which is consistent with the earlier appearance of tardy condensation observed in figures 1 and 2. The diffusion of chromatin within the accessory discloses to observation a small, black, spherical granule lying close to the vesicular membrane at its proximal end (stain, Heidenhain's iron-hæmatoxylin). This granule is always seen in the accessory in this stage and always at the polar end. The ordinary chromosomes in figure 4 do not show a similar element, owing to their slight degree of internal dissociation, but in the later stages, figures 6, 7, 9 and 10, its presence is clearly demonstrated.

The slight shifting of position of the chromosomes, due to the diffusion of the chromatin and the enlargement of the chromosomal vesicles, presents a difficulty when it is attempted to determine the location of the granules. Sections of cells may be cut in all conceivable planes and many misleading figures obtained. The sections in which the least complication occurs, and the ones best fitted for study, are those in which the entire accessory is visible in longisection lying at one side of the nucleus. (See fig. 6.) Here there can be no doubt as to which are the polar ends of the chromosomes, and their limits can be ascertained, showing that the polar ends of all of the chromosomes do not lie in the same plane but that the deeply staining round granules which mark their polar ends lie near the same side of the nucleus. It is also noteworthy that these black bodies occur only at the polar ends. Other chromatin masses which are observed occasionally at other points within a chromosome are indistinct in outline, irregular in shape, and cannot be shown to be constant, so are not to be confused with the polar granules.

In cross-section of these diffused stages we obtain such figures as 7, in which we are looking down on the proximal ends of the chromosomes, and in figure 8, which is a section through the central or distal portion of the chromosomal group. Figure 8 shows the stage midway between two succeeding metaphases in which the dissociation of chromomeres is at its maximum, and is present in the same degree in all of the chromosomes. Here too the polar granules prove constant in occurrence, staining qualities and position.

The two stages immediately following the one shown in figure 9 must be studied side by side to interpret correctly the nature of the changes which take place. In figure 10 the change seems to be merely a closer aggregation of chromatin particles in which a transverse striation is noticeable. Comparing this with the following condition, seen in figure 11, we can plainly see that the beginning of the prophase involves the organization of the chromatin into a much convoluted thread.*

The manner in which the condensed homogeneous elements viewed in figure 1 are formed from the long, loosely organized spirals of figure 13 is shown by a comparison of figures 10 to 15. With the beginning of the prophase, figure 10, the contiguous walls of certain vesicles disappear, resulting in a formation of a few (actual number uncertain) large non-intercommunicating vesicles each containing a number of rods showing a longitudinal split. The accessory in figure 14 does not exhibit this division. The entire disappearance of the chromosomal vesicles and the arrangement of the chromosomes in the equatorial plate, figure 1, marks the completion of the series of changes comprising one spermatogonial division.

In the telophases of the last spermatogonial division the changes are somewhat different from those just described. The chromosomal vesicles, with the exception of that surrounding the accessory chromosome, disappear with the diffusion of the chromatin and the nucleus becomes spherical in shape. The accessory seems to vary in the amount of diffusion that it undergoes but is usually observed as a slender black-staining rod closely appressed to the nuclear wall.

FIRST SPERMATOCYTE.

The diffused chromatin of the last spermatogonial telophase is found in the early spermatocyte prophase to be reorganized into a number of filiform segments which show a characteristic arrangement. The accessory chromosome, a shapeless mass of homogeneous chromatin, lies at one side of the cell (fig. 16 x). Near it, and next to the nuclear wall, is a group of bodies resembling the accessory in all but size, being much smaller. From these the chromatic threads extend outward, each segment apparently forming a loop the ends of which have their termination in the smaller chromatin bodies. A. and K. E.

* The author was unacquainted with Bonnevie's work, "Chromosomen Studien I, Archiv für Zellforschung," Bd. 1, Heft 2-3, which appeared after this was written, and which describes similar changes.—C. E. M.

Schreiner, in their studies of *Spinax niger* and *Myxine glutinosa*, have described a looped arrangement of the thread during the conjugation period similar to figures 16 and 17. Here conjugation takes place by the lengthwise union of entire threads, each chromomere of one uniting with a homologous chromatin unit of the other, thus forming a thicker chromatin thread with a longitudinal division. No such union of threads as they describe has been observed in my material. The division in the thread precedes that in the polar granule, and often we find several polar granules united with the radiating threads showing a longitudinal split (figs. 19, 20 *a*). Here again we have a resemblance to one of the figures of the above authors, in which they find the chromatin threads radiating from a chromatic body which they call the "Knotenkörper." Figure 19 shows the accessory chromosome with no threads attached.

By a subsequent contraction and longitudinal splitting of these threads in figure 16, tetrads like those shown in figure 18 are formed. The interesting and peculiar feature of these formations are the condensed bulbous thickenings marking the synaptic end of each chromatid. These changes have taken place within the nuclear membrane. The accessory has assumed a regular form and still maintains its lateral position. The lesser bodies, grouped as represented in figures 16 and 17, are missing unless we identify them with the condensed thickenings on the ends of the chromatids. As the chromatids coalesce the longitudinal divisions of the thread and its homogeneous ends are obliterated, and we find many figures resembling figure 18*f*. Oftentimes these peculiar enlargements occur at each end of a chromatid. This is confusing and, in the light of my limited observations, unaccountable. Figures 21 to 27 show the usual succession of changes in the first spermatocyte division. There are twelve chromosomes in the metaphase. Figures 22 and 23 show the constancy of form which prevails within the species. There are always two large rings, six smaller rings, two very short rods, and one longer rod. The accessory, homogeneous throughout the prophases, becomes rough in outline during the brief moment of its journey to the pole, but regains its smooth contour in the telophase of the first spermatocyte (fig. 28). A slightly later telophase shows the accessory less condensed but apparently

not increased in size. A like change in the ordinary dyads now shows granules in their synaptic ends staining similarly to those previously noted in the spermatogonial chromosomes. (See fig. 29.)

SECOND SPERMATOCYTE.

The ordinary dyads in a prophase of the second spermatocyte resemble those in figure 28. The condition of the accessory was not noted. Division proceeds in the usual manner, resulting in the separation of single chromatids (figs. 30-32). Figure 33 shows a cell formed by this latter division. Diffusion of the chromatids has taken place within the nucleus. Persisting bodies of chromatin are noted distributed irregularly through the less concentrated nuclear substance. A round, deeply staining mass of spindle fibers is observed adjoining the nucleus (fig. 33 *a*). After the stage represented in figure 33 we obtain figure 34, a late stage in the developing spermatid. All traces of material staining like condensed chromatin have disappeared with the exception of the middle piece (fig. 34 *a*). It is uncertain whether this body lies within or without the membrane *b*.

CONCLUSIONS.

In conclusion, I wish briefly to summarize and correlate the history of the unusual chromosomal elements noted in the four generations of germ-cells just described. I have already referred to those occurring in the spermatogonial chromosomes as the polar granules. There their prominence and constancy claim for them recognition as important elements in the organization of the individual chromosomes. By a consideration and comparison of figures 4, 6 and 9 it is plain that the position of these granules coincides with the proximal ends of the chromosomes, and evidently, from figure 6—which shows that the granules occupy that point of the chromosome which is nearest the pole—mark the point of attachment of the spindle fiber. If now we can prove that these granules maintain the same position with respect to the remaining portion of the chromosomal substance throughout its life-history, and also that every such granule is the direct descendant of a preceding granule exactly like it in form and position, we may, I think, safely conclude that every chromosome is definitely and unchangeably polarized and that the point of spindle fiber at-

tachment is constant. In view of the lateral position of the polar granules in the telophases of the spermatogonia and their proximity to the accessory chromosome, I am convinced that in the small bodies of condensed chromatin in figures 16 and 17, showing early spermatocyte prophases, we have the direct product of two conjugating polar granules. Each polar granule contains material from each chromatid, and consequently, in the tetrads of the first spermatocyte, when the division between chromatids is apparent, we have from the union of two polar granules four bulbous thickenings at the synaptic ends of the tetrad, which indicate also the location of the spindle fiber attachment. Through the processes which follow, these centers of condensation prove permanent elements, reappearing in the dyads of the ensuing telophase (fig. 29). From these observations it appears that the polar granules are permanent bodies, not undergoing marked physical change during the processes of the cell division. This permanency of position, in the case of the polar granule, seems to indicate the existence of a force which governs the relative position of the constituent elements of the chromosome through its various changes. Such a function may be ascribed to the linin thread which undoubtedly forms an important part of every chromosomal entity.

The occurrence of condensations at the distal ends of the chromatids occasionally observed in the spermatocyte tetrads cannot be explained by the preëxistence of similar condensations in the spermatogonial chromosomes, for such are lacking there.

It would be interesting as well as instructive if we could determine definitely the function of these polar granules. Unquestionably they are in some way concerned with the definite polarization of the complex element to which they belong, and their relations both to the spindle fiber and to synapsis is significant. In consideration of this question Doctor McClung has suggested a comparison of the polar granule in the spermatogonial chromosomes with the body from which the axial filament of the spermatid grows. The analogy consists in the fact that in both cases we have a small, definitely formed mass of homogeneous chromatin located at one end of an elongated membranous vesicle and marking the attachment of a movable filament, but we are unable to judge the meaning of these

similarities, since we have no evidence as to the fate of the polar granules in the developing spermatid. I am inclined to believe that the bodies observed in figure 33 are the persisting polar granules, but whether these undergo further dissolution or unite to form the middle piece of the mature spermatid must be left for decision by future investigations.